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DISCUSSION

ARTHUR TOGA (*UCLA School of Medicine, Los Angeles, Calif.*): Given the fact that a lot of the anatomic nomenclature has been defined historically prior to the use of the chemo-architectural visions you have shown us, do you think that a lot of the nomenclature and the way in which we catalog or describe brain is going to be influenced by these emerging and lovely ways of looking at brain?

APPEL: I don't know whether they will, but I hope so. It is very hard to sit down with the old anatomy books and the new anatomy books and then go to a meeting and know whether you are talking about the same thing as the presenter. I would like the nomenclature to evolve with the additional information we get.

QUESTION: Do you know of any work where an attempt was made to make better dye? I am thinking of the *in vitro* stain that you alluded to. Has something been made that could be used for analysis of an aldehyde-fixed postmortem tissue, which, of course, would help us understand human neuroanatomy much better.

APPEL: The dye I referred to is called Fluoro-Jade. It is a fluorescent stain used to reveal degenerating neurons and their processes. The staining technique was developed by Dr. Larry Schmued of the FDA National Center for Toxicological Research. The paper is in press in the journal *Brain Research*.

Assessment of Neurotoxicity from Potential Medications for Drug Abuse: Ibogaine Testing and Brain Imaging

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The resistance of drug abuse disorders to existing therapies underscores the need for new medications for treatment. Pharmacological actions of such drugs in the central nervous system (CNS) can be assessed using technologies developed in a variety of disciplines, ranging from anatomy and biochemistry through psychology and medical physics. The development of the hallucinogen ibogaine as a potential therapeutic agent will serve to illustrate the approaches used to elucidate toxic effects in laboratory animals and the proposed assessment of analogous deficits in human subjects.

THE IBOGAINE EXAMPLE

In 1991, the Medications Development Division of the National Institute on Drug Abuse initiated a project to evaluate the toxicity of *Tabernanthe iboga* (ibogaine) as a prerequisite for clinical trials with this agent in cocaine-dependent human volunteers. Ibogaine is extracted from the roots of an *apocynaceous* shrub, which is indigenous to and used in religious rites in west central Africa, mainly Gabon and Zaire.¹ The drug acts as a stimulant at low doses and as a hallucinogen at high doses. In human subjects, the hallucinogenic threshold is approximately 300 mg.² Higher doses (6 to 25 mg/kg) reportedly reduced cocaine craving for periods of weeks to months.^{1,3}

Preclinical studies have indicated that ibogaine reduced the self-administration of morphine (0.04 mg/kg unit dose)⁴ and cocaine (0.4 mg/kg unit dose) in the rat.^{5,6} The effects lasted for 24 hours or several days in some animals.⁵ Some laboratories have failed to replicate the effect on self-

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administration of cocaine in the rat,⁷ and others could not demonstrate it in the rhesus monkey.⁸

The mechanism of putative anti-addictive properties of ibogaine is unknown, as the drug interacts with several neurotransmitter systems in the CNS. Radioligand binding studies have shown that, at concentrations in the micromolar range, ibogaine binds to the phencyclidine site on the *N*-methyl-D-aspartate (NMDA) receptor complex; to *mu*, *kappa*, and *delta* opioid receptors;⁹⁻¹² to dopamine, norepinephrine and serotonin uptake sites;¹¹ and to Na⁺ channels.⁹ Of particular interest, however, is a possible effect of ibogaine on mesocortical and mesolimbic dopaminergic systems, as these systems have been identified as critical to the rewarding properties of drugs of abuse.¹³ Cell bodies which originate in the ventral tegmental area project to the medial prefrontal cortex, nucleus accumbens, and amygdala. When administered acutely to rats, most drugs of abuse (amphetamine, cocaine, morphine, nicotine, and ethanol) increase levels of extracellular dopamine in the nucleus accumbens of the brain.^{13,14} Amphetamine and cocaine also increase dopamine levels in the prefrontal cortex.^{15,16}

Studies using *in vivo* microdialysis have revealed that ibogaine, given alone, increases extracellular levels of dopamine in the prefrontal cortex, but not the nucleus accumbens in rats.¹⁷ Moreover, ibogaine influences the effects of morphine and psychomotor stimulants on extracellular dopamine levels, although the response to ibogaine in this regard is not universal. Whereas ibogaine (40 mg/kg) reduced the morphine-induced increase in extracellular dopamine¹⁸ and blocked increases in motor activity usually produced by morphine (up to 20 mg/kg, i.p.),¹⁹ the drug potentiated the rise in extracellular dopamine levels produced by cocaine (20 mg/kg, i.p.) or amphetamine (1.25 mg/kg, i.p.) in the rat and potentiated the motor response.^{19,20}

Standard toxicology studies of ibogaine in the rat revealed that doses above 25 mg/kg produced a constellation of CNS signs, including ataxia, splayed hindlimbs, outstretched forelimbs, Straub tail, and hyperexcitability.²¹ In addition to these effects on overt behavior, the drug also had remarkable cerebellar actions. Three doses of ibogaine (100 mg/kg, i.p.) enhanced the expression of glial markers, such as glial fibrillary acidic protein (GFAP), in cerebellar tissue.²² An increase in GFAP immunoreactivity in narrow radial bands of the cerebellar vermis was likely due to activation of Bergmann glial cells. Astrocytic processes extended through the molecular and Purkinje cell layers and the outer part of the granule cell layer. Microglia labeled with cytochemical markers, such as OX42 (for the CR-3 receptor), OX6 (for the MHC II complex), and W3/25 (for the rat CD4 receptor), were activated in the cerebellar cortex of the vermis of ibogaine-treated rats, with a distribution similar to that seen with GFAP. The increase in astrocytic and microglial markers suggested Purkinje cell loss. Indeed, further work showed that a single dose of ibogaine (100 mg/kg, i.p.) produced losses in Purkinje cells, as demonstrated by the Gallyas reduced silver staining method for degenerating neurons, and reduced binding of molecular markers, such as

microtubule-associated protein 2 and calbindin D₂₈.²³ An example of the characteristic neurotoxic effect of ibogaine on Purkinje cells in rat cerebellum is shown in FIGURE 1. Purkinje cells were stained with an antibody to calcium-calmodulin-dependent protein kinase II (Cam KII), a histochemical marker for this cell type in the cerebellar vermis.²³ In this instance, a single dose of 100 mg/kg ibogaine produced Cam KII-negative, parasagittal stripes, indicating neuronal loss. The pattern of damage extended through the Purkinje cell and molecular layers of the vermis and the paravermis, and longitudinal bands reflected cell loss ranging from 1 to 10 cells per band.²⁴

Increases in GFAP, measured by an enzyme-linked immunosorbent assay, were noted in several brain regions of female rats after administration of oral ibogaine (25–150 mg/kg) for 14 days.²⁵ GFAP concentrations were elevated in the hippocampus and brain stem of rats given 25 mg/kg, and in olfactory bulb and cerebral cortex of animals treated with 150 mg/kg. A differential sensitivity to ibogaine was noted between genders, with females showing greater sensitivity.

GFAP levels also were above control values in several brain regions of female dogs following a single dose of ibogaine (100 mg/kg).²⁶ Involvement at several levels of the neuraxis, from the cerebral cortex to the brainstem, was inferred, suggesting a potentially more widespread phenomenon than that seen in the rat. Histopathological studies were not performed in the dog brain.

Ibogaine neurotoxicity in the rhesus monkey was evaluated following i.p. administration of 50 mg/kg every 3 to 4 hours for 12 hours.²⁷ Tremor, ataxia, myoclonus, and grand mal seizures were observed 5–10 min after ibogaine administration. Although activated microglia and neuronal cell loss were observed, the degree of both was small compared to that observed in rat cerebellum.

Data generated in preclinical studies are used to assess the relative safety of administration of investigational agents to human subjects. When toxicities are noted, decisions are made either not to begin a clinical study or to proceed and monitor the human subjects for possible similar adverse events. The data summarized above suggested that ibogaine could induce behavioral effects and variable neurotoxicity in rats, dogs, and monkeys. Therefore, the NIDA group designing the proposed clinical study of ibogaine was justifiably concerned about toxicity of ibogaine in the cerebellum and other regions of brain. They devised an approach for evaluating potential neurotoxicity, with baseline measurements of deficits prior to ibogaine administration constituting the initial step. The protocol proposed to evaluate several cerebellar measures, using both clinical and computer-assisted measurements of postural sway. Cognitive measures using standard and computer-driven test batteries were to be assessed at baseline and at specified intervals following a single ibogaine administration.

A related clinical example of drug-induced cerebellar degeneration has been known for almost 40 years, as cerebellar degeneration is a hallmark toxicity of chronic ethanol abuse.²⁸ Moreover, a recent study showed that



FIGURE 1. Coronal section of the cerebellum of a rat administered a single dose of ibogaine, 100 mg/kg, i.p. Purkinje cells were stained with antibodies to CAM-kinase II. Light areas represent zones of Purkinje cell degeneration resulting from ibogaine treatment. Degeneration occurs in narrow bands in the sagittal plane.²³

long-term alcohol intake (41–80 grams per day for 20–30 years) produced an average loss of 33 percent of Purkinje cells in 66 subjects evaluated at autopsy.²⁹ Only 4.5 percent of the subjects had macroscopic signs of degeneration. In another clinical series, 78 chronic alcoholics were evaluated neurologically and via posturographic measurements on a force-measuring platform. Clinical signs of cerebellar ataxia were noted in 33 percent of the subjects whereas posturographic abnormalities were seen in 69 percent.³⁰ Taken together, these results suggest that Purkinje cell damage may occur in heavy drinkers/alcoholics and that posturographic measures may be a more sensitive indicator of damage than clinical examination.

IMAGING PROCEDURES TO ASSESS ACUTE AND CHRONIC TOXICITY DUE TO TREATMENT MEDICATIONS

New technologies utilized for monitoring function can be more sensitive than clinical examination for the assessment of desired or undesired effects. A variety of noninvasive imaging procedures have become available to study the structure and function of the human brain. These procedures have not been used primarily in assessments of potential untoward effects of treatment medications; however, their use in other areas of neuroscience illustrates how they can be applied in the development of new medications for the treatment of drug abuse. These techniques include nuclear medicine procedures, such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) as well as structural and functional magnetic resonance imaging (MRI). In some instances, noninvasive functional imaging can reveal an abnormality years before clinical signs develop. In others, changes seen may be compensated for through system reserves, redundancy, or plasticity.

The technologies used for monitoring may focus on a particular neurotransmitter system (e.g., assay of binding to a specific neurotransmitter receptor) or they may assess structural or functional integrity of a variety of brain regions (e.g., MRI or metabolic mapping with PET). Pharmacological challenges may be used to unmask changes that may be undetected in an unperturbed system. Furthermore, simultaneous application of several assay instruments, including behavioral, electrophysiological and nuclear medicine approaches, may be appropriate and useful for establishing correlations between changes in specific aspects of brain function and amelioration of a disease (drug abuse disorder) or its sequelae. The assay method selected for safety assessment depends on the effect(s) of concern, generated in preclinical testing or observed in patients in previous studies. Similarly, the same types of sophisticated technology can be applied to evaluate proposed changes in brain function, as parameters of efficacy.

One of the most important principles in the evaluation of potential neurotoxicity is the pre-existing condition of the patient/subject. Chronic substance abuse can produce subtle deficits which can be revealed using sophisticated technologies, such as MRI and PET. Studies in polydrug abusers

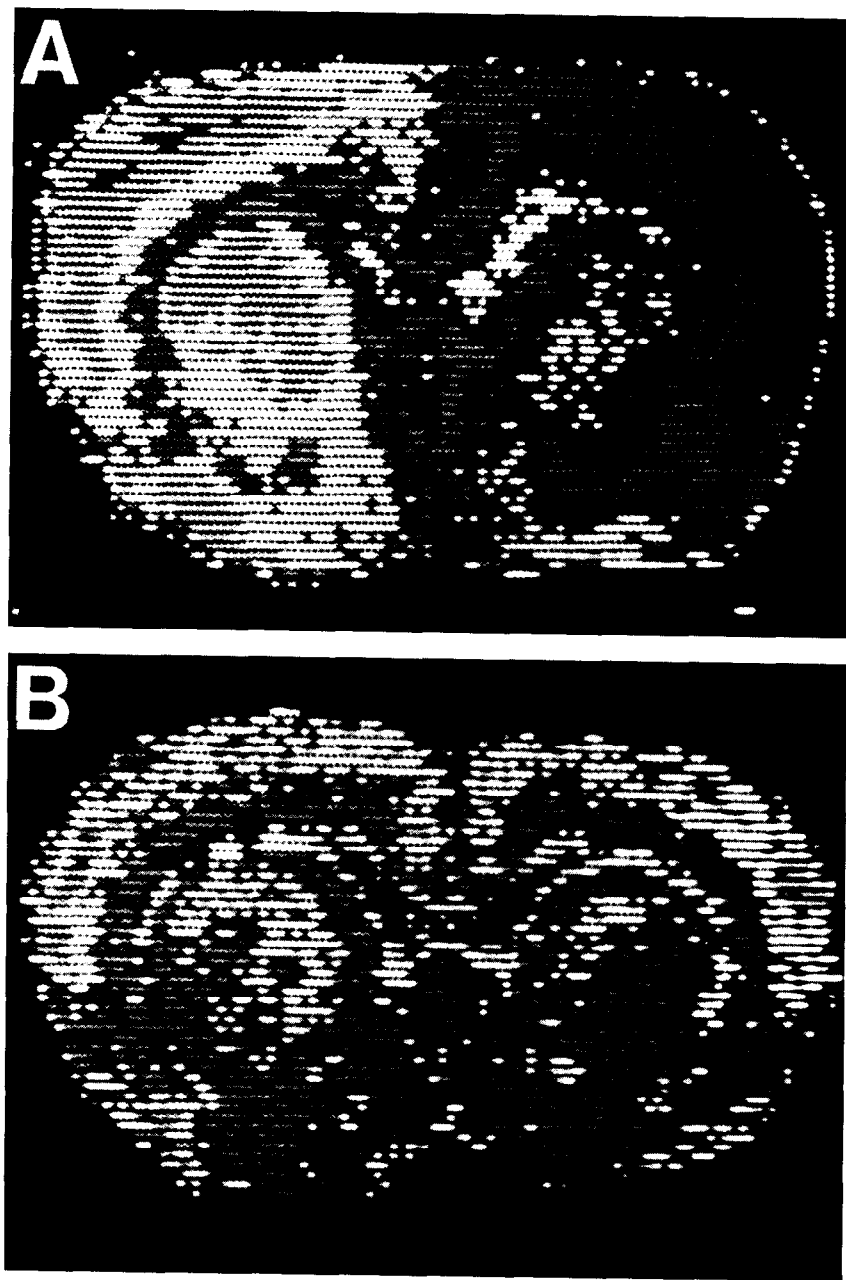


FIGURE 2

have demonstrated the relative to greater sensitivity of functional nuclear medicine procedures as compared with structural imaging in assessing the effects of chronic drug abuse. PET assays of human volunteers using [F-18]fluorodeoxyglucose (FDG) have demonstrated that individuals with histories of polydrug abuse, including intravenous substance abuse, have an abnormal pattern of cerebral glucose metabolism as compared with controls matched for age and socioeconomic status.³⁰ This abnormality is seen in subjects who have been abstinent from illicit drugs of abuse for up to one month, and consists of a deficit in glucose metabolism in the visual association cortex and abnormally high relative metabolic rate in the orbitofrontal cortex. In addition, deficits in cerebral perfusion have been observed in chronic cocaine abusers, using PET³² and SPECT.³² Such differences are not readily apparent when individuals drawn from the same populations are compared with respect to a general measure of structural integrity of the brain, such as the ventricle-to-brain ratio.³⁴ Volumetric MRI of a group of polydrug abusers revealed no tendency for ventriculomegaly in a group of polysubstance abusers as compared with controls using volumetric MRI scanning. Nonetheless, assays of particular brain regions have revealed differences between polydrug abusers and controls. Substance abusers show significantly smaller volume of the prefrontal lobe, containing the orbitofrontal region,³⁵ which shows abnormal metabolism on PET scans.³¹ Whether or not this difference reflects the effects of chronic drug abuse or hypoplasia, which may have contributed to development of substance abuse disorder, is unknown at this time.

Greater sensitivity of functional measures (e.g., obtained by PET) as compared with structural assays has been documented in the early days of PET scanning. A classic example was presented in the study of Huntington's disease. Serial studies of an individual who ultimately developed the clinical syndrome of Huntington's disease revealed that prior to the manifestation of clinical neurological signs, roentgenographic computerized tomography (CT) scanning revealed no abnormality in the basal ganglia, while glucose metabolism in the basal ganglia was severely reduced.³⁶

In addition to assays of brain function, as inferred from rates of glucose metabolism and regional cerebral blood flow, neurochemical integrity has

FIGURE 2. Transforms of autoradiograms from 20- μ m brain sections from rats subjected to the 2-deoxy-D-[1-¹⁴C]-glucose ([¹⁴C]DG) procedure. Dark gray values represent high levels of grain density; light values represent low levels of grain density. (A) Autoradiogram of a brain section from a representative rat in which a lesion was induced by ibotenic acid (12 μ g) three days prior to the [¹⁴C]DG procedure. (B) Autoradiogram of a brain section from approximately the same level of a representative rat "lesioned" with ibotenic acid (12 μ g) 28 days before being subjected to the [¹⁴C]DG procedure. In (A), note the reduction in grain density, representing decreased regional cerebral metabolic rate for glucose in portions of the autoradiogram corresponding with the frontoparietal cortex and striatum of the lesioned hemisphere. In (B), there was virtually no asymmetry in grain density of cortical regions, although the density was somewhat lower in the striatum of the lesioned side.⁴⁰

been assessed primarily by receptor binding assays. Although most receptor binding assays up to this point have attempted to quantitate available receptor number or receptor density in various disease states or in normal physiological conditions, nuclear medicine procedures, such as SPECT and PET, have more recently been considered for assessment of neurotransmitter dynamics and interactions between various neurotransmitter systems.³⁷ For example, in light of the interaction between cholinergic and dopaminergic systems in brain, assays of ¹¹C-raclopride binding to D2-like dopamine receptors in response to pharmacological perturbation can reflect changes in intrasynaptic dopamine as influenced by the action of an anticholinergic agent.³⁸ Changes in intrasynaptic dopamine in response to the indirect agonist amphetamine have been observed using SPECT and ¹²³I-iodobenzamide.³⁹ Given the preclinical findings of cerebellar changes induced by ibogaine, assays of non-dopaminergic systems in cerebellum may be of great interest.

LONG-TERM CONSEQUENCES OF NEUROTOXICITY: ADAPTATION

Studies in animals have suggested that neurochemical changes in brain that might be induced by toxins or potential medications may persist without functional impairment, as the brain adapts to a chronic neurotransmitter deficiency. One example of this type of situation is presented in rats that received unilateral local injection of the glutamatergic neurotoxin, ibotenic acid, into the ventral globus pallidus.³⁵ In such rats, the neurotoxin causes degeneration of cells of the nucleus basalis magnocellularis, which provides the primary cholinergic innervation to the neocortex. Animals treated with such intracerebral injections showed severe depression in the concentration of choline acetyltransferase, the synthetic enzyme for acetylcholine, and no recovery over a 5-week period.⁴¹ Nonetheless, animals that manifested marked deficits in cortical glucose metabolism within one week of the lesion showed complete recovery and no asymmetry one month after placement of the lesion.⁴⁰ Similarly, rats subjected to unilateral nucleus basalis lesions showed a return of choline uptake to control values, with a gradual increase, reaching normal levels after 3 months.⁴² These observations indicate that even after substantial neurotoxin-induced lesions, the brain can show significant plasticity over time. The extent to which a history of drug abuse might preclude recovery after treatment with a potentially neurotoxic agent is not known.

SUMMARY

New technologies utilized for monitoring brain function can be more sensitive in the assessment of desired or undesired pharmacological effects than can clinical examination. Nonetheless, careful case-by-case analysis is

required to determine to what extent a change detected with a sensitive imaging modality will have clinical significance. Whereas in some instances the technology may suggest a subclinical condition years before clinical signs develop, in other instances changes seen may be compensated for through system reserves, redundancy, or plasticity. Furthermore, simultaneous application of several assay instruments, including behavioral, electrophysiological, and nuclear medicine approaches, may be appropriate and useful for establishing correlations between changes in specific aspects of brain function and amelioration of a disease (drug abuse disorder) or its sequelae. In the example of ibogaine, a testing strategy was developed to assess human subjects for possible changes in cerebellar function (that were suggested by preclinical findings indicating subtle damage). Thus, subjects may be tested for subclinical alterations during and immediately following a clinical trial. This "harbinger of toxicity" approach would provide clinicians the critical data necessary for appropriate follow-up of subjects as well as the propriety of continuance of the clinical trials within the ibogaine project.

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DISCUSSION

QUESTION: With regard to the ibogaine, do you know whether there is any histopathology in the hippocampal area, given its effects on the excitatory amino acid receptors?

VOCCI: Apparently there isn't. There is *C-fos* activation and GFAP elevation at 25 mg/kg in forebrain areas, but no apparent histopathology that we are aware of.

QUESTION: Could you comment on the specificity of effects of drugs that apparently operate via similar mechanisms, such as the glutamate receptors?; because another chemical, domoic acid, has substantial effects on hippocampal areas and rather limited effects on cerebellar regions.

VOCCI: That may be because of the fact that the effect on the cerebellum is a transsynaptic one rather than a direct effect. Each drug may have a different mechanism, which may provide the clue to the type of toxicity you see and the region you see it in.

QUESTION: With regard to the nucleus basalis magnocellularis region, although one shows that blood flow or glucose metabolism becomes symmetrical in a chronic lesion, you can see upregulation of postsynaptic M1 regulative signal transduction involving phospholipase A2 *in vivo*, with

radiolabeled arachidonic acid. So despite the fact that the M1 receptor density is the same in the cortex after M2 has been removed 2 weeks after a lesion has been made, one can in fact see a neuroblastic response at the level of signal transduction *in vivo* with autoradiography and inference both with PET.

VOCCE: Yes.

QUESTION: What is the neuropharmacopathology of vincristine? Is it the same as oubain? Also, have these same studies been done with cytarabine, which oncologists probably see as a more common cause of ataxia.

VOCCE: I believe that the evidence for neuropathology from vincristine is clinical. I am not sure that anyone has actually looked at whether or not vincristine damages Purkinje cells, but it is an obvious place to look. As for cytarabine, I believe that a generic cytarabine that was on the market several years ago actually produced some cerebellar lesions. All cytarabine will do it. The question is whether this generic compound was more likely to do it than the innovator.

Imaging Studies of Cocaine in the Human Brain and Studies of the Cocaine Addict^a

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Positron emission tomography (PET) is a medical imaging modality that measures the regional distribution and kinetics of chemical compounds labeled with short-lived positron-emitting isotopes in the brain and living body.¹ Several radiotracers have been developed that enable the measurement of various aspects of brain neurochemistry and function. Of the PET tracers developed, the one most utilized is 2-deoxy-2-[18F]-fluoro-D-glucose (FDG). FDG is an analogue of glucose that enables the measurement of regional brain glucose metabolism. Because brain regional glucose utilization is tightly associated with brain function,² the measurement of FDG with PET has allowed the assessment of brain activity in human subjects.

The availability of PET tracers to monitor specific neurotransmitter systems has made it possible to directly evaluate them *in vivo* in human subjects and provides a tool to investigate their contribution in the pharmacological properties of drugs as well as its disruption with chronic drug use and abuse. Various elements pertaining to neurotransmitter function can be investigated with PET. For example, one can assess receptors subtypes, as has been done with the dopamine D1 and the dopamine D2 receptors. Elements from the presynaptic terminal can be directly evaluated by using tracers that assess metabolism of neurotransmitters, such as is the case for L-DOPA, and/or by monitoring monoamine transporters. The concentration of enzymes in brain that are involved with degradation of monoamines has also been achieved with the development of ligands that bind to MAO-B, MAO-A and COMT.³

Since positron emitters can be used to label compounds without affecting their pharmacological behavior, PET can also measure drug pharmacokinetics *in vivo* in brain as well as in various organs throughout the body. Because these studies are done in living subjects, the relation between pharmacokinetics and the temporal course of the behavioral effects of drugs can be investigated.

The relatively short half-life of positron emitters and the relatively benign

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